THE PRESENCE OF THE TRICARBOXYLIC ACID CYCLE IN THE CILIATE COLPIDIUM CAMPYLUM [1]

GERALD R. SEAMAN [2, 3]

The Marine Biological Laboratory, Woods Hole, Mass. and the Biological Laboratory, Fordham University, New York

Colpidium campylum is a ciliate which can be easily cultured bacteria-free in a liquid medium. It has been demonstrated that when cultured in proteose-peptone from which the lipids have been extracted, the organism is capable of synthesizing large amounts of fatty acids (Wilber and Seaman, 1948).

Since the tricarboxylic acid cycle is a link between protein and carbohydrate metabolism, it seemed desirable to make a study of this cycle as the first step toward the elucidation of the pathway for fatty acid synthesis from protein in this organism.

While there have been many investigations of this cycle in vertebrate tissue and in bacteria, there has been little done with protozoa. Van Niel, Thomas, Ruben and Kamen (1942) found that the ciliate *Tetrahymena geleii* assimilates carbon dioxide in the anaerobic formation of succinate during the fermentation of glucose. Baker and Baumburger (1941) found cytochrome c, b, and a_1 to be present in this same organism with indications of the presence of cytochrome a_2. Hutchens, Jandorf and Hastings (1941) ascertained the DPN content of the flagellate *Chilomonas paramecium.* Hutchens (1940) also identified the presence of cytochrome c in Chilomonas. Laurie (1935) demonstrated the presence of succinic dehydrogenase in the ciliate *Glaucoma pyriformis.*

Materials and Methods

Colpidia were grown in sterile, pure cultures in 150 cc. Erlenmeyer flasks containing 50 cc. of 3 per cent Difco proteose-peptone solution from which the carbohydrate had been precipitated with copper sulfate (Peters and Van Slyke, 1931) and the lipids extracted with hot alcohol (Bloor, 1943). The organisms used were obtained from cultures maintained in the Biological Laboratory, Fordham University and are the same strain as was used in a previous investigation (Wilber and Seaman, 1948). For use in this investigation, new cultures were inoculated with 1 cc. of organisms from a three-day culture and allowed to grow for two days at a temperature of $22 \pm 2^\circ$ C. At this time the cultures were at the mid-point of the logarithmic phase of growth (population about 40,000 colpidia per cc.).

The organisms for use were concentrated by centrifugation and aliquots with-

[1] Portion of a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Fordham University.

[2] The author wishes to express his sincere gratitude to Dr. Charles G. Wilber for invaluable aid and encouragement.

[3] U. S. Public Health Service Fellow.

drawn for ascertaining the dry weights of the cells (Ormsbee, 1942). The remaining cells were washed three times with Hahnert's solution (Hahnert, 1932), to which was added magnesium sulfate to make a final concentration of 0.02 M (final pH adjusted to 5.6). The cells were then starved for twelve hours before use. At the end of this period the organisms were again concentrated, resuspended in the modified Hahnert's solution and 2 cc. portions (containing approximately 10 mg. dry weight of cells) transferred into standard Warburg vessels.

Oxygen uptake was measured by the conventional Warburg direct method. In all cases the total volume of each vessel was 3.5 cc. Vessels were shaken at a rate of 120 cycles per minute through an arc of 5 cm.

Sodium pyruvate was prepared by the method of Robertson (1942); oxaloacetic acid by the method of Krampitz and Werkman (1941). All other substrates were obtained commercially. Concentrations of substrates are given as final concentration.

Pyruvic and α-ketoglutaric acids were estimated according to the method of Friedmann and Haugen (1943); succinic acid according to Krebs (1937); oxaloacetate according to Edson (1935); fumaric acid according to Krebs, Smyth and Evans (1940).

Results

Pyruvate is rapidly metabolized by Colpidium. When 0.02 M pyruvate is added to cells respiring in modified Hahnert's solution there is an immediate

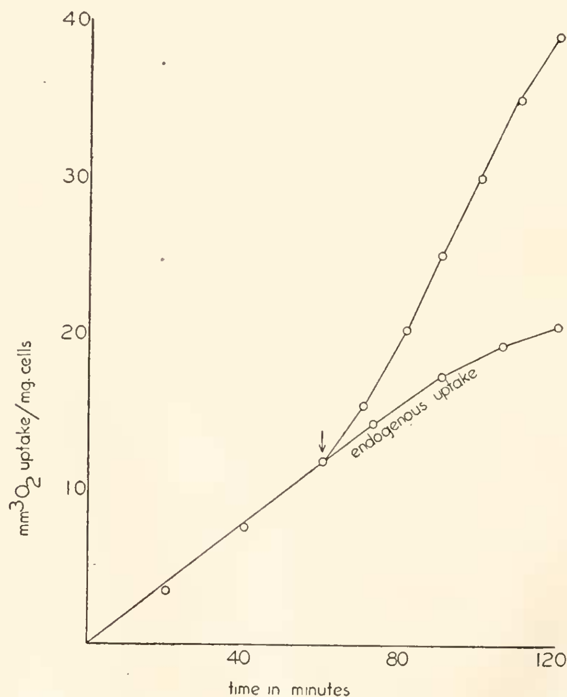

FIGURE 1. Effect of pyruvate on oxygen uptake in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. At arrow, 0.02 M pyruvate added.

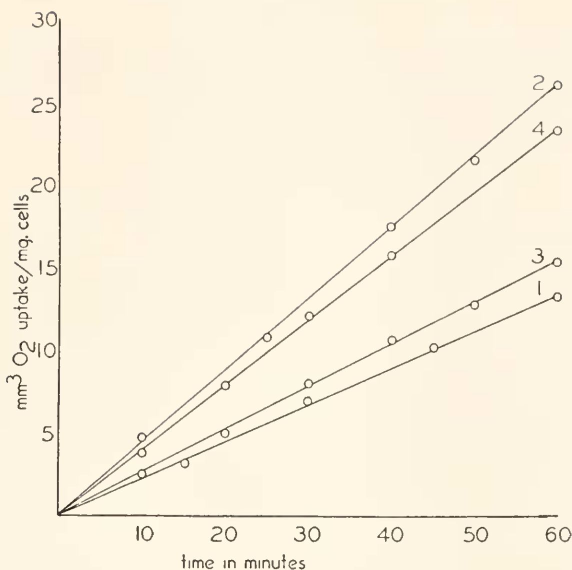

FIGURE 2. Effect of malonate and fumarate on oxygen uptake in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. Curve 1, no added substrate; curve 2, 0.02 M pyruvate; curve 3, 0.02 M pyruvate + 0.02 M malonate; curve 4, 0.02 M pyruvate + 0.02 M malonate + 0.001 M fumarate.

increase in the rate of oxygen uptake (Fig. 1). There is a utilization of 0.081 mg. of pyruvate per mg. dry weight of cells per hour (Table II).

If the tricarboxylic acid cycle plays a role in the metabolism of Colpidium, the oxygen uptake in the presence of pyruvate should be inhibited by malonate. This inhibition should be released upon the addition of fumarate. Figure 2 shows that 0.02 M pyruvate increases the Q_{O_2} from the endogenous value of 13.2 to 26.3, an increase of 99 per cent. In the presence of pyruvate and 0.02 M malonate the Q_{O_2} is 15.4, 83 per cent inhibition of the pyruvate effect. The Q_{O_2} is restored to a value of 23.6 by the addition of 0.001 M fumarate, an 89 per cent recovery of the malonate inhibition.

The effect of other acids of the tricarboxylic acid cycle on oxygen uptake is shown in Table I. Succinate results in an increased Q_{O_2} of 105 per cent; α-ketoglutarate 102 per cent; fumarate 90 per cent; malate 97 per cent; and oxaloacetate, an increase of 85 per cent.

The quantities of metabolites recovered from various substrates are shown in Table II and III. Fumarate and α-ketoglutarate are recovered in approximately equal amounts when pyruvate is the substrate. The addition of fumarate to pyruvate increases the recovery of α-ketoglutarate by 142 per cent. Fumarate and pyruvate are recovered in a ratio of approximately 1 to 4 when oxaloacetate is utilized as a substrate.

Table III shows that as a result of the fumarate release of malonate inhibition, there is an added utilization of 0.051 mg. pyruvate per mg. dry weight of cells per hour, and an added recovery of succinate amounting to 0.013 mg. per mg. dry weight of cells per hour.

TABLE I

Effect of acids of the tricarboxylic acid cycle on oxygen uptake in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. Concentration of all substrates except fumarate, 0.02 M; fumarate, 0.001 M.

Q_{O_2}	Substrate
13.2	—
27.1	succinate
26.8	α-ketoglutarate
25.1	fumarate
26.1	malate
24.5	oxaloacetate

TABLE II

Utilization of substrates and recovery of intermediate metabolites in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. $Q_{substrate}$ is mg. substrate utilized (−) or mg. metabolite formed (recovered) (+) per mg. dry weight of cells per hour. Pyruvate, 0.02 M; oxaloacetate, 0.002 M; fumarate, 0.001 M.

$Q_{substrate}$	Substrate added		
	pyruvate	pyruvate + fumarate	oxaloacetate
$(+)_{pyruvate}$	—	—	0.044
$(-)_{pyruvate}$	0.081	0.065	—
$(+)_{ketoglutarate}$	0.012	0.029	—
$(-)_{oxaloacetate}$	—	—	0.121
$(+)_{fumarate}$	0.016	—	0.014

TABLE III

Formation of succinate in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. Malonate, 0.02 M; pyruvate, 0.02 M; fumarate, 0.001 M.

$Q_{substrate}$	Substrate added (in addition to malonate)	
	pyruvate	pyruvate + fumarate
$(-)_{pyruvate}$	0.016	0.067
$(+)_{succinate}$	0.003	0.016

TABLE IV

Effect of succinate, α-ketoglutarate, and citrate in releasing malonate inhibition in Colpidium. Modified Hahnert's solution, pH 5.6. Gas phase, O_2. Temperature, 25.5° C. Malonate, pyruvate, α-ketogluterate, succinate, 0.02 M; citrate, 0.008 M; fumarate, 0.001 M.

Substrate (in addition to pyruvate which was present in all vessels)	Q_{O_2}
— — — —	26.3
malonate	15.4
citrate	26.1
citrate + malonate	13.7
citrate + malonate + fumarate	22.8
α-ketoglutarate + malonate	28.4
succinate + malonate	27.6

Added citrate in final concentrations ranging from 0.002 M to 0.01 M has no effect on the oxygen uptake. Table IV shows the ability of citrate in releasing malonate inhibition as compared to the ability of succinate and α-ketoglutarate to release the inhibition. Succinate and α-ketoglutarate release the malonate inhibition to approximately the same extent as does fumarate (compare Fig. 2), whereas citrate does not release the inhibition.

Discussion

It would be desirable to compare the Q_{O_2} values obtained for Colpidium in this investigation with values obtained for other protozoa. However, it is impossible to make such a comparison, since it was found (Ormsbee, 1942) that the Q_{O_2} of the same species of Tetrahymena varies from 6.2 to 77.7 depending upon the age of the culture, the length of the starvation period before oxygen uptake is measured, and the composition of the suspending medium. Other factors affecting Q_{O_2} values in protozoa are the rate of shaking of the manometer vessels (Hall, 1938) and the concentration of cells used (Pace and Lyman, 1947). Hutchens (1941) found that in *Chilomonas paramecium* the oxygen uptake per hour per 10,000 cells varies with different strains, even though both strains are studied under identical conditions.

Since added citrate does not increase oxygen uptake or release malonate inhibition, and since fumarate, succinate, and α-ketoglutarate do cause increased oxygen uptake and do release malonate inhibition, it must be concluded (Stare, Lipton, and Goldinger, 1941) that citrate does not occupy a major position in the tricarboxylic acid cycle as it occurs in Colpidium.

It appears from the data of Von Dach (1942) that the tricarboxylic acid cycle is not present in the colorless flagellate, Astasia. In this organism, succinate, fumarate and malonate have no significant effect on the oxygen uptake. In *Paramecium caudatum,* succinate increases oxygen uptake by only 8 per cent (Leichsenring, 1925).

Elliott (1935) found that pyruvic acid (0.5%) inhibits growth in *Colpidium campylum* and in *C. striatum.* On the other hand, Bond (1933) found that pyruvic acid stimulated growth in *C. campylum.* However, he found that succinate (1.0%) and malate (1.0%) inhibit growth. These findings are unusual if, as has been demonstrated in this paper, these compounds are metabolites. It must be noted that the concentrations used by these authors were very much higher than those used in the present investigation. It is well known that normally occurring metabolites in high concentrations may cause inhibition of metabolic functions, as measured by oxygen uptake. It would be desirable to ascertain the effects of acids of the tricarboxylic acid cycle on the growth of Colpidium when used in concentrations which are known to be physiologically active (0.001–0.02 M). Such an investigation is now in progress.

Summary

1. Evidence is presented for the presence of the tricarboxylic acid cycle in the metabolisms of the ciliate *Colpidium campylum.*

2. Apparently citrate does not occupy a major position in the tricarboxylic acid cycle as it occurs in Colpidium.

LITERATURE CITED

BAKER, E. S. G. AND J. B. BAUMBURGER, 1941. The respiratory rate and cytochrome content of a ciliate protozoan (Tetrahymena geleii). *Jour. Cell. Comp. Physiol.*, **17**: 285–303.

BLOOR, W. R., 1943. Biochemistry of the fatty acids. Reinhold, N. Y., 387 pp.

BOND, R. M., 1933. A contribution to the study of the natural food cycle in aquatic environments. *Bull. Bing. Ocean. Coll.*, **4**: 1–89.

EDSON, N. L., 1935. Ketogenesis-antiketogenesis. I. The influence of ammonium chloride on ketone-body formation in liver. *Biochem. Jour.* **29**: 2082–2094.

ELLIOTT, A. M., 1935. Effects of certain organic acids and protein derivatives on the growth of Colpidium. *Arch. f. Protis.*, **84**: 472–494.

FRIEDMANN, T. E. AND G. E. HAUGEN, 1943. Pyruvic acid. II. The determination of keto-acids in blood and urine. *Jour. Biol. Chem.*, **147**: 415–442.

HAHNERT, W. F., 1932. A quantitative study of reactions to electricity in Amoeba proteus. *Biol. Bull.*, **80**: 265–274.

HALL, R. H., 1938. The oxygen consumption of Colpidium campylum. *Biol. Bull.*, **75**: 395–408.

HUTCHENS, J. O., 1940. The need of Chilomonas paramecium for iron. *Jour. Cell. Comp. Physiol.*, **16**: 265–267.

HUTCHENS, J. O., 1941. The effect of the age of the culture on the rate of oxygen consumption and the respiratory quotient of Chilomonas paramecium. *Jour. Cell. Comp. Physiol.*, **17**: 321–332.

HUTCHENS, J. O., B. J. JANDORF, AND A. B. HASTINGS, 1941. Synthesis of diphosphopyridine nucleotide by Chilomonas paramecium. *Jour. Biol. Chem.*, **138**: 321–325.

KRAMPITZ, L. O. AND C. H. WERKMAN, 1941. The enzymatic decarboxylation of oxaloacetate. *Biochem. Jour.*, **35**: 595–602.

KREBS, H. A., 1937. The role of fumarate in the respiration of Bacterium coli commune. *Biochem. Jour.*, **31**: 2095–2124.

KREBS, H. A., D. H. SMYTH, AND E. A. EVANS, 1940. Determination of fumarate and malate in animal tissues. *Biochem. Jour.*, **34**: 1041–1045.

LAURIE, N. R., 1935. Studies on the metabolism of protozoa. II. Some biochemical reactions occurring in the presence of the washed cells of Glaucoma pyriformis. *Biochem. Jour.*, **29**: 2297–2302.

LEICHSENRING, J. M., 1925. Factors influencing the rate of oxygen consumption in unicellular organisms. *Am. Jour. Physiol.*, **75**: 84–92.

ORMSBEE, R. H., 1942. The normal growth and respiration of Tetrahymena geleii. *Biol. Bull.*, **82**: 423–437.

PACE, D. M. AND E. D. LYMAN, 1947. Oxygen consumption and carbon dioxide elimination in Tetrahymena geleii. *Biol. Bull.*, **92**: 210–216.

PETERS, J. P. AND D. D. VAN SLYKE, 1931. Quantitative clinical chemistry. Vol. 2. Methods. Williams and Wilkins, Baltimore.

ROBERTSON, W. B., 1942. The preparation of sodium pyruvate. *Science*, **96**: 93–94.

STARE, F. J., M. A. LIPTON, AND J. M. GOLDINGER, 1941. Studies on biological oxidation. XVIII. The citric acid cycle in pigeon muscle respiration. *Jour. Biol. Chem.*, **141**: 981–987.

VAN NIEL, C. B., J. O. THOMAS, S. RUBEN, AND M. D. KAMEN, 1942. Radioactive carbon as an indicator of carbon dioxide utilization. IX. The assimilation of carbon dioxide by protozoa. *Proc. Nat. Acad. Sci.*, **28**: 157–161.

VON DACH, H., 1942. Respiration of a colorless flagellate, Astasia klebsii. *Biol. Bull.*, **82**: 356–371.

WILBER, C. G. AND G. R. SEAMAN, 1948. The lipids in Colpidium campylum. *Biol. Bull.*, **94**: 29–32.